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The preservative efficacies of bemul-wax coatings in combination with calcium chloride dip on low temperature stored *Citrus O. sinensis*

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Abstract

The capacity of developing countries for citrus fruits exportation is limited by the lack of adequate storage techniques. In studying this problem *citrus sinensis Osbeck* was treated with, a locally developed bemul-wax, calcium chloride dip, and a combination of the two. The treated fruits were assessed during four month low temperature storage (7.0 ± 3.0^{o}C; 90-94% RH.) for their biochemical and sensory qualities. Fruits treated with bemul-wax and the combination manifested significantly reduced ($p<0.05$) physiological weight, delayed ripening, and texture values. All the treated oranges showed significantly reduced titratable acidity. Combined treatment significantly reduced ($p<0.05$) ascorbic acid level, while the combined and calcium chloride dip treatments reduced, and increased significantly ($p<0.05$) the density of the juice respectively. All treatments showed significantly increased ($p<0.05$) pH and α-amylase activities but no effects on total sugar levels, pectin esterase activities, taste and flavor of the juice. In conclusion, the combined and bemul-wax treatments preserved the nutritional, biochemical, and sensory qualities of the stored oranges for the four month storage period considered adequate to cover the orange off-season period in Nigeria as well as sufficient to export orange from Nigeria to other parts of the world.

Keywords: Sweet orange, Bemul-wax, Calcium chloride dip, Combination, Low temperature storage

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INTRODUCTION

Climacteric fruits may be exported in the unripe preclimateric state so that it can be of a longer storage life, greater resistance to damage and still having the capacity to ripen to a good quality through the correct temperature and possibly ethylene management[1]. Post harvest losses of fruits and vegetables in Nigeria had been reported[2] to be 50% due to improper handling, packaging, transportation, and storage particularly due to hot and humid weather. Cold storage is not often used due to its high capital investment. Under tropical ambient conditions, fruits ripen rapidly after harvest at the green mature stage, becoming soft in texture and predisposed to injury[3]. Appropriate technology to extend the shelf life and reduce post harvest losses of oranges is therefore required in order to exploit the market potential that exists.

Calcium in plant is usually found in the cell wall forming calcium bridges between residues of galacturonic acid belonging to adjacent pectic chains[4,5]. Undoubtedly, our capability to manipulate calcium effects in postharvest systems will be enhanced as the understanding of calcium action advances in the future[5].

Simple waxing is also a technique that could probably be used during storage more widely in developing countries with advantages. In some countries indigenous waxes may be suitable for this purpose. In Colombia, it has been shown that waxing of cassava can extend the storage life from 2-3 days up to about 30 days by preventing discoloration in the vascular tissue[6]. An indigenous wax emulsion "Bemul-wax" was developed from cassava starch[7] for shelf life extension of agro-produce, which will consequently promote exportation in Nigeria.

The use of bemul-wax had earlier been reported for storage of mandarin orange[7], and sweet potato[8]. The combination of these two techniques (calcium chloride dip and bemul-waxing) has not been used for storage of oranges before. This work was designed to examine the biochemical and sensory effect of these treatments on the stored oranges, and to recommend the best treatments for citrus fruits during storage.

MATERIALS AND METHODS

Sample Preparation

Four hundred and fifty oranges were harvested from a fifteen year old orange tree in an orchard farm of Lower Niger River Basin Development Authority, Ilorin, Kwara State. Nigeria.

The matured wholesome ones of about 244.15 ± 35.71 g and 40-50% color break (i.e. amount of orange color often associated with ripening) were sorted out and divided into four equal lots containing 100 oranges each; two lots were washed with clean water and later dipped in 20mM calcium chloride solution for 30mins. One of the two lots, Calcium chloride dip treated sample (CCDL) was allowed to dry while the second lot, combined treated sample (COML) was afterward coated with bemul-wax (100% concentration). Another lot Bemul-wax coated sample (BWL) was coated with bemul-wax alone after washing with clean water, while the 4th lot (unwashed) served as control (CL). BWL coated oranges were allowed to dry under ventilation for thirty minutes. The oranges were thereafter stored at 7.0 ± 3.0°C; 90-94% Relative Humidity (RH) for four months in a temperature and humidity controlled refrigerator.

The weights of five labeled oranges in each lot were taken at monthly interval for four months to assess the physiological loss in weight (PLW). Juices from three oranges were extracted at each interval for determination of pH, titratable acidity, density, ascorbic acid, total sugar, alpha amylase and pectin esterase activities.

Three replicates were used for each analysis. The results were also subjected to statistical analysis using ANOVA statistical software package[9].Weight of 10ml of juice was taken and the resultant weight obtained was afterwards divided by the volume to determine the density of each juice. 20ml of each juice was put in a beaker, and the pH was thereafter taken using Jenway model 3310 pH meter.

Titratable acidity was assayed using the method of A.O.A.C.[10]. The results were expressed as percentage citric acid content of the juice. Total sugar content was extracted by method described by Southgate[11] and analysed spectrophotometrically[12]. The ascorbic acid was extracted and analysed spectrophotometrically[13-15]. Pectin methyl esterase (PME) was determined by the extraction and assay method[16]. The α-Amylase extraction and activity was determined as described by Okolie and Ugochuckwu[17,18].

Sensory evaluation

Five oranges were taken from all the treatments and control, and displayed for sensory observation. The whitish portion (albedo) of oranges from each lot was removed to obtain only the pulp segments.

These were chosen randomly for assessing the taste and flavor of the juice at each interval. Ten trained panellists were used to assess the products on a 10 point hedonic scale. Water was taken by the panellists in between each sample to prevent possible influence of the previous taste on the next.

The ratings were graded as 0-2 "poor" , 3-4 "fair", 5-6 "good", 7-8 "very good", and 9-10 "excellent". The fruits were assessed for skin orange color, texture, taste, flavour, and overall quality.

RESULTS

Bemul-wax coating and the combined treatments significantly reduced ($p<0.05$) physiological loss in weight (Fig 1) throughout the four months storage, the reduction occurred only at the first month of storage for calcium chloride dip treated oranges. The pH of all the treated oranges were significantly lower ($p<0.05$) during the storage period compared with control (Table 1).

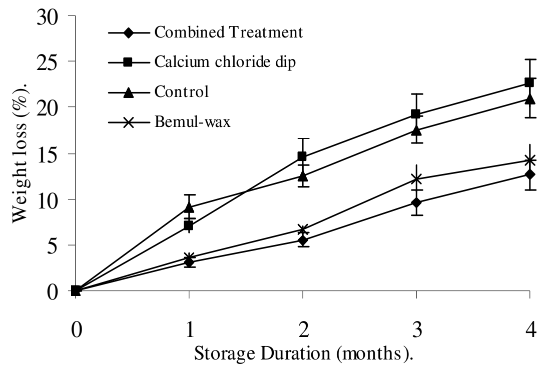

Fig. 1: Physiological loss in weight of sweet oranges treated with bemul-wax, calcium chloride and a combination of both during low temperature storage (7.0±3.0°C; 90-94% RH)

Table 1: Biochemical Qualities of Treated Oranges during Storage

Biochemical Parameters	Treatment	Period of Storage (Months)				
		0	1	2	3	4
pH	Cl	4.04 ± 0.04	5.91 ± 0.00	4.36 ± 0.00	4.32 ± 0.03	4.75 ± 0.01
	COML	4.04 ± 0.04	3.98 ± 0.01	4.41 ± 0.00	4.54 ± 0.01	4.32 ± 0.06
	CCDL	4.04 ± 0.04	4.11 ± 0.01	4.22 ± 0.00	4.19 ± 0.05	4.22 ± 0.02
	BWL	4.04 ± 0.04	4.91 ± 0.00	4.16 ± 0.00	4.43 ± 1.40	4.29 ± 0.03
Titratable acidity (%citric acid)	Cl	0.72 ± 0.02	0.86 ± 0.01	0.83 ± 0.02	1.05 ± 0.01	0.81 ± 0.00
	COML	0.72 ± 0.02	0.73 ± 0.01	1.01 ± 0.01	0.81 ± 0.01	0.82 ± 0.03
	CCDL	0.72 ± 0.02	0.81 ± 0.00	0.82 ± 0.01	0.98 ± 0.01	0.82 ± 0.02
	BWL	0.72 ± 0.02	0.80 ± 0.02	0.85 ± 0.00	0.80 ± 0.01	0.93 ± 0.00
Total sugar (mg/g fresh juice)	Cl	14.86 ± 3.03	17.71 ± 0.45	21.82 ± 3.44	9.73 ± 0.81	11.58 ±0.03
	COML	14.86 ± 3.03	26.79 ± 8.48	21.82 ± 0.56	11.78 ± 0.34	11.75 ±1.07
	CCDL	14.86 ± 3.03	11.33 ± 6.28	20.89 ± 1.37	11.41 ± 0.46	11.46 ±0.06
	BWL	14.86 ± 3.03	24.83 ± 6.37	21.24 ± 0.91	11.06 ± 3.12	11.13 ±0.06
Ascorbic acid (μg/g juice)	Cl	213.33±11.25	386.04±68.72	242.53±26.15	255.76±38.78	343.36±30.76
	COML	213.33±11.25	420.32±18.34	124.08±38.31	277.80±66.94	120.77±23.92
	CCDL	213.33±11.25	347.88 ± 75.48	223.25±4.16	254.11±19.09	250.25±10.72
	BWL	213.33±11.25	430.77 ± 32.84	224.90±11.01	251.35±47.49	162.65±31.41

COML(Combined treated samples, CCDL (Calcium chloride dip treated samples), BWL (Bemul-wax coated samples)

Table 2: Biochemical Qualities of Treated Oranges during Storage

Biochemical Parameters	Treatment	Period of Storage (Months).				
		0	1	2	3	4
Alpha Amylase activity (µmol/min./g fresh wt.)	Cl	1.318± 0.568	1.019± 0.245	1.820± 0.245	2.561± 0.255	1.931± 0.091
	COML	1.318± 0.568	0.553± 0.343	1.220± 0.488	2.150± 0.379	1.904± 0.340
	CCDL	1.318± 0.568	1.039± 0.427	1.474± 0.170	2.307± 0.190	1.953± 0. 348
	BWL	1.318± 0.568	0.741± 0.308	1.851± 0.052	2.078± 0.131	1.915± 0.322
Pectin esterase activity (µmol/min./g fresh wt.)	Cl	9.575± 0.005	9.578± 0.008	9.545± 0.321	9.555± 0.033	9.466± 0.076
	COML	9.575± 0.005	9.564± 0.016	9.544± 0.167	9.563± 0.159	9.469± 0.079
	CCDL	9.575± 0.005	9.580± 0.009	9.553± 0.061	9.575± 0.009	9.514± 0.034
	BWL	9.575± 0.005	9.555± 0.045	9.567± 0.006	9.505± 0.030	9.526± 0.008
Density (g/ml)	Cl	1.032± 0.003	1.032± 0.002	1.029± 0.001	1.039± 0.006	1.039± 0.001
	COML	1.032± 0.003	1.031± 0.002	1.029± 0.001	1.033± 0.001	1.033± 0.001
	CCDL	1.032± 0.003	1.031± 0.001	1.032± 0.005	1.036± 0.004	1.044± 0.002
	BWL	1.032± 0.003	1.031± 0.004	1.031± 0.001	1.036± 0.003	1.037± 0.002

COML(Combined treated samples, CCDL (Calcium chloride dip treated samples), BWL (Bemul-wax coated samples)

The combined treatment significantly reduced (p<0.05) ascorbic acid level at the end of storage, while significant (p<0.05) reduction and increase in density of the juice manifested in the combined treatment and the calcium chloride dip treated fruits respectively at four month storage (Table 2). Both the combined treatment and calcium chloride dip treatment significantly reduced (p<0.05) the titratable acidity (Table 1) during the storage period. Bemul-wax treated oranges however became significantly higher (p<0.05) in titratable acidity at the end of storage.

Significantly higher (p<0.05) alpha amylase activities was manifested in all the treated oranges (Table 2) only at one month storage. The activities however generally increased as storage progressed. No significant effect (p>0.05) of treatment were observed on total sugar (Table 1), and pectin esterase activity (Table 2) throughout the duration of storage although pectin esterase activities for all treated fruits and control were generally decreasing as storage progressed.

Sensory Evaluation

The sensory qualities of the stored sweet oranges are as shown in Table 3. Bemul-wax and the combined agents significantly delayed (p<0.05) ripening as indicated by the retention of the green color during the first-three months storage. Also, oranges treated with bemul - wax, and the combined agents were significantly better (p<0.05) in texture values than control during the storage duration. A significant reduction (p<0.05) in taste of the control fruits occurred at third month of storage.

No significant effect (p>0.05) of treatments on the flavor of the juice, and overall quality of the fruits were generally observed during the storage period.

Table 3: Sensory Qualities of Treated Oranges during Storage**.**

Sensory Parameters	Treatment	Period of Storage (Months)				
		0	1	2	3	4
External orange colour	Cl	4.5 ± 2.0	7.8 ± 1.0	7.9 ± 2.1	8.8 ± 1.0	7.9 ± 2.2
	COML	4.5 ± 2.0	5.5 ± 1.7	5.7 ± 2.3	5.0 ± 1.2	7.8 ± 1.4
	CCDL	4.5 ± 2.0	7.0 ± 1.1	6.2 ± 1.1	8.6 ± 0.7	7.8 ± 2.0
	BWL	4.5 ± 2.0	5.0 ± 1.6	6.1 ± 1.7	3.4 ± 1.0	6.6 ± 1.5
Texture	Cl	6.2 ± 2.0	7.1 ± 1.5	5.8 ± 1.8	4.4 ± 1.1	4.3 ± 1.3
	COML	6.2 ± 2.0	6.2 ± 1.8	7.6 ± 2.0	6.8 ± 1.7	7.2 ± 1.3
	CCDL	6.2 ± 2.0	5.8 ± 1.9	5.0 ± 1.6	3.2 ± 1.7	4.8 ± 2.0
	BWL	6.2 ± 2.0	7.6 ± 2.3	7.5 ± 1.8	7.3 ± 2.7	8.4 ± 1.7
Taste	Cl	6.9 ± 2.0	7.2 ± 1.5	5.9 ± 2.2	5.7 ± 2.4	6.1 ± 2.0
	COML	6.9 ± 2.0	6.2 ± 1.8	7.0 ± 1.6	5.5 ± 1.4	5.7 ± 1.3
	CCDL	6.9 ± 2.0	6.5 ± 1.7	6.3 ± 1.6	5.1 ± 1.7	5.3 ± 1.6
	BWL	6.9 ± 2.0	7.1 ± 1.3	7.5 ± 1.4	7.6 ± 0.8	7.2 ± 1.3
Flavor	Cl	7.1 ± 1.5	7.2 ± 1.6	6.2 ± 1.9	5.7 ± 2.1	6.3 ± 1.6
	COML	7.1 ± 1.5	7.1 ± 1.5	7.1 ± 1.6	5.9 ± 1.4	5.5 ± 1.8
	CCDL	7.1 ± 1.5	6.7 ± 1.6	6.4 ± 1.3	4.8 ± 1.8	5.9 ± 1.8
	BWL	7.1 ± 1.5	7.1 ± 1.4	7.0 ± 1.6	7.2 ± 1.2	7.0 ± 2.2
Overall quality	Cl	7.3 ± 1.2	8.1 ± 1.3	6.6 ± 2.0	5.3 ± 2.2	5.2 ± 2.6
	COML	7.3 ± 1.2	6.8 ± 1.6	7.5 ± 1.7	6.6 ± 1.5	6.3 ± 2.4
	CCDL	7.3 ± 1.2	6.9 ± 1.3	6.4 ± 1.5	4.8 ± 1.4	6.4 ± 1.7
	BWL	7.3 ± 1.2	7.8 ± 1.4	7.7 ± 1.4	7.7 ± 1.3	6.4 ± 2.9

COML(Combined treated samples, CCDL (Calcium chloride dip treated samples), BWL (Bemul-wax coated samples)

DISCUSSION

Both bemul-wax coatings and the combined treatment prevented water loss that often leads to losses in appearance (e.g. wilting and shriveling), texture, and nutritional composition[19]. The mass per unit volume (densities) of the juices were generally increased as storage progressed, which could be due to the effect of moisture loss through evaporation.

The increase in alpha amylase activity during this low temperature storage is similar to earlier report[20]. De novo synthesis was suggested for the increase[21]. The increase in amylase activity is a reflection of increased sugar level and sweetness[22], which may be as a result of the need to generate more glucose to sustain the energy required by the tubers[23], or for the production of polyphenols required to sustain the fruits defence mechanisms. The utilization of glucose to sustain the physiological requirement of the tubers may be attributed to the general decrease in sugar levels shown in Table 1.

Both the combined treatment, and bemul-wax coatings could improve the economic power of orange traders in Nigeria since the fruit weight loss was prevented by both treatments at the end of storage. The estimate cost of oranges in Nigerian local market during the year 2005 was put at thirty two thousand Nigerian Naira (₦32,000.00) per ton[24]. The use of calcium chloride dip alone may not be recommended beyond one month's storage since it could not prevent weight loss beyond one month.

The pH of the juice ranged between 4.035-5.910, 4.035-4.535, 4.035-4.220, and 4.035-4.43 for the control oranges, combined treatment, calcium chloride dip and bemul-wax treated oranges respectively.

This work shows that calcium chloride dip affect titratable acidity and pH reported in treated fruits (Table 1) may be due to the delayed ripening in such fruits since there are more organic acids in unripe fruits[25] that may be generated by increased activity at the tricarboxylic acid. The low titratable acidity (Table 1) observed in both calcium chloride dip, and the combined treated fruits can be attributed to the influx of calcium ions into the oranges[26].

The titratable acidity of the stored oranges ranged between 0.72-1.053%, 0.72-0.814%, 0.72-0.982%, 0.72-0.934% for control, combined treated, calcium chloride dip, and bemul-wax treated oranges respectively. These values were considered within acceptable range of 0.6-1.0% reported for oranges[27]. Since acidity imparts sour taste, and is an important attribute in the acceptability of citrus fruits.

The density of the juice was not affected until fourth month of storage. This is an indication of the onset of deterioration of the quality of the juice in control orange and calcium chloride dip treated orange. Similar to the report of Sting and Rouseff[25], the total sugar levels in control, and treated fruits increased up to the second month of storage. This may be due to the gradual increase in alpha amylase activity observed in this study (Table 2).

The activities of the pectin esterase enzyme were not inactivated by the treatments. Thus, continuous deterioration of texture may not be arrested. It may also be implied that the preserved texture observed during sensory evaluation (Table 3) may not be totally attributed to enzymatic action but more importantly on the ability of the treatments to prevent moisture loss.

The external orange color of the oranges usually an indicator of its ripening level generally increased as the storage progressed. The oranges were about 4.5 % ripened (i.e. not fully ripped) at harvest. The control and calcium chloride dip treated oranges ripened faster during storage. Bemul-wax coating and that of the combined treatments delayed ripening for a period of four, and three months respectively.

All the sensory parameters of the harvested oranges were rated “good”. The textures however generally appreciated for only one month in control, but for four months in both the combined treated oranges, and bemul-wax coated oranges. The calcium chloride dip treatment was only able to retain the texture for two months. The superiority of bemul-wax coatings alone in improving the texture, followed by that of the combined treatments is highly demonstrated in this study. The improved texture observed in Table 3 for treated fruits may not be attributed to the ability of treatments to eliminate pectin esterase activity but ability to prevent physiological loss in weight since our treatment did not affect the enzyme activity.

Taste was retained for one months in control, two months in both the combined treated oranges, and calcium chloride dip treated oranges, but four months in bemul-wax coated oranges. Depreciation began to set in after these stages, but the taste were however generally acceptable for all the treatment and control throughout the storage period. This report indicated that bemul-wax (contrary to earlier report on waxes) does not lead to off taste of the stored orange.

Flavor was retained for one month in both control and calcium chloride dipped oranges, two months in combined treated oranges, and four months in bemul-wax coated oranges. However, the flavor for all the treatments and control were generally acceptable throughout the storage duration.

The overall quality was retained for two months in both control, and the combined treated fruits, one month in calcium chloride dip treated oranges, and four months in bemul-wax coated oranges. The overall quality for all the treated oranges and control were however generally acceptable throughout the storage duration.

The lack of effect of treatments on flavor also confirms that no fermentation (normally experienced in wax coated fruits) occurred as a result of these treatments (especially bemul-wax). The development of off flavors had earlier been associated with wax coated fruits[28-30]. Both bemul-wax and the combined treated oranges were adjudged even better during the first-three months of storage.

This report indicats that all the treated fruits quality were within the following recommended quality standards (relative densities of 1.045-1.055, ascorbic acid of 200mg/fruit, minimum sensory score qualities of 6.0 on 10point scale (or 5.0 point on 8pt scale) for color and appearance, and 5.0 point on 10point scale for aroma and flavor) reported for oranges[25].

In conclusion, the biochemical and sensory qualities of the oranges at harvest were preserved in the combined and bemul-wax treated oranges during the four month storage. This four month storage period is considered adequate to cover the orange off-season period in Nigeria as well as sufficient to export orange from Nigeria to other parts of the world. The combination of bemul-wax coatings and calcium chloride dip delayed ripening of sweet orange for four months with better nutritional and sensory qualities. This is recommended for orange treatments. The treatment could also save traders from economic loss through adequate reduction of weight loss.

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